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Original Article

Partial incubation and its function in great tits (*Parus major*)—an experimental test

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In many bird species, females incubate the eggs for short periods before clutch completion. The role of such partial incubation and the effective egg temperatures attained are controversial. Possible functions depend on whether temperatures reach the critical values required for embryogenesis and include the protection of the clutch from predators, brood parasites, and environmental factors; inhibition of bacterial growth on eggs; control of hatching asynchrony; or energy saving by parents. In this study, in a natural population of great tits, we first investigated the occurrence and extent of partial incubation, and second, we evaluated some potential functions by experimental simulation of the observed patterns of partial incubation. We found that egg temperatures during partial incubation were clearly raised above the physiological zero temperature for embryo development. Simulation of partial incubation did not affect any breeding parameters or nestling morphological traits compared with controls. It suggests that partial incubation with temperatures above the minimum temperature for embryo development does not serve to increase the variation in nestling morphology and thus competitiveness, in particular, does not increase hatching asynchrony within brood as one of its currently most discussed functions. *Key words:* egg temperatures, hatching asynchrony, maternal effects, nestling morphology, *Parus major*, phenotypic variation. *[Behav Ecol]*

INTRODUCTION

Maternal effects are a powerful mechanism to influence development and phenotype of offspring and to promote adaptation to complex and changing environments (Mousseau and Fox 1998). They occur in both plants (e.g., Schwaegerle and Levin 1990; Montalvo and Shaw 1994) and animals (e.g., Parichy and Kaplan 1992; Bernardo 1996) and are especially important in species where females make a considerable investment in offspring (Reinhold 2002).

In birds, parents may use incubation behavior to influence offspring development and phenotype (Howe 1976; Wiebe, Korpimaki, et al. 1998; Vinuela 2000; Krist et al. 2004) because embryonic development in avian eggs requires a minimum temperature. This physiological zero temperature has been found to be between 25 and 27 °C in several species (Drent 1975). Incubation behaviors are complex and varied among species and in general, 2 modes have been identified. Partial incubation occurs in almost 50% of the species (Wang and Beissinger 2011) and is characterized by short bouts of incubation mostly at night during egg laying. Full incubation, in contrast, is characterized by more constant egg temperatures close to 34–36 °C (mean internal egg temperature, Drent 1975; Haftorn 1981; Wang and Beissinger 2011) until hatching.

The extent and causes of partial incubation are poorly understood and little is known about the temperatures attained during this behavior (Wang and Beissinger 2011). Birds may attend to the eggs without raising their temperature or actively sit on the eggs and cause a marked temperature increase. Partial incubation without a raise of temperature above physiological zero may 1) function as a protection against egg predators or brood parasites (Wiebe and Martin 1998; Clotfelter and Yasukawa 1999) or 2) protect eggs from precipitations, moisture, solar rays (Morton and Pereyra 1985), freezing, or lethal heat (Ward 1990). It may also simply prevent nest site takeover by other potential breeders (Beissinger et al. 1998) and finally reduce thermoregulatory costs for parents (Pendlebury and Bryant 2005).

Partial incubation with heat transfer above physiological zero temperature can serve several functions. The viability of unincubated eggs declines over time (Arnold et al. 1987; Veiga 1992) and partial incubation can reduce the risk of hatching failure (Webb 1987; Meijerhof 1992; Veiga 1992) because embryos that have passed the early developmental stages can be more resistant to environmental fluctuations, as shown in some domestic species (Fasenko 2007). Also a rise in egg temperature may lead to lyzosyme activation, an enzyme in the albumen with antimicrobial proprieties (Wellman-Labadie et al. 2008; Ruiz-de-Castaneda et al. 2012). Additionally, if partial incubation may contribute to embryo development, this in turn may increase the developmental differences among embryos in a clutch laid over several days and in turn increase hatching asynchrony (Davies and Cooke 1983; Kennamer et al. 1990). Initiation of embryo development by partial incubation may reduce the duration of full incubation and the length of the nestling period and hence affect the timing of egg laying (Wang and Beissinger 2011). Partial incubation with heat transfer may be costly for the female (Morton and Pereyra 1985; Deeming 2002).

Full incubation (also called active or effective incubation, e.g., Wang and Beissinger 2011) starts often already before the clutch is completed (Meijer and Siemers 1993; Beissinger and Stoleson 1997; Wiebe, Wiehn, et al. 1998; Loos and Rohwer 2004). It is assumed that the variation in the start of full incubation plays a fundamental role for hatching asynchrony and hatching patterns (Wiebe, Korpimaki, et al. 1998; Wang and Beissinger 2009) with short- and/or long-term consequences for offspring growth and development

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(Slagsvold 1986; Slagsvold et al. 1995; Mainwaring et al. 2012), fledging success (Hebert and McNeil 1999), postfledging survival, and local recruitment (Amundsen and Slagsvold 1998; Cam et al. 2003; Laaksonen 2004). Onset of active incubation with respect to the laying sequence can influence parental body condition and survival (Slagsvold et al. 1995; Hanssen et al. 2002), affect a female's clutch size, and reduce the risk of predation for both offspring and adults (Wang and Beissinger 2009).

The purpose of this study was to 1) investigate the occurrence and extent of partial incubation and 2) evaluate its function by experimentally simulating the observed patterns of partial incubation. The study was performed in a free-living population of great tits (*Parus major*), a hole-breeding small Passerine species where the occurrence and function of partial incubation above physiological zero temperature is currently disputed (Haftorn 1981; Pendlebury and Bryant 2005; Lord et al. 2011).

METHODS

The study was conducted between March and May 2010 and 2011 in the Könizbergwald, a forest near Bern, Switzerland (46°56′N, 7°24′ E): in 2010, to evaluate the occurrence of partial incubation; and in 2011, to test its function. From early March each year, nest-boxes were visited daily to determine laying date and clutch size. To investigate the patterns of incubation during the early egg-laying stages, in 2010, we measured egg temperature using data loggers (I-button, UK). We placed the data loggers in the middle of the nest cup between the eggs. Data loggers were programmed to record the temperature every 5 s. The accuracy of the data loggers was tested in an incubator in the lab at 3 different temperatures: 10, 25, and 33 °C and recorded over 30 min every 5 s. All data loggers appeared to be highly accurate ($N = 40$, mean temperature ± 1 SD: for 10 °C = 10.5 °C ± 0.17; for 25 °C = 25.2 °C ± 0.26; for 33 °C = 32.9 °C ± 0.30). Data loggers were inserted inside 36 randomly chosen nests between the day the first egg was laid and the first day of full incubation. Data loggers have the shape of a flat round button with a diameter of 17.5 mm and a thickness of 6 mm weighing ~2 g (great tit eggs are oval though quite variable in shape, around 17 × 13 mm and weigh ~1.7 g). Because of their flat shape, the buttons will most of the time remain underneath the eggs. Indeed, when checking nest-boxes, we have never observed a logger on top of the eggs, but always underneath. Thus, it is unlikely that females are in direct contact with the buttons via the brood patch and it is hence likely that our estimate of egg temperatures is rather conservative and may be slightly warmer than indicated by the logger. There was no nest desertion after the data loggers were inserted. None of the data loggers was discarded from the nests during the laying period, as may be expected if females were disturbed by the presence of the logger. Moreover, it seems that females turned the eggs as usual because data loggers changed their position in the nest cup over time. Six females discarded the logger at the start of full incubation.

Thirteen females were caught in 2010 when nestlings were 12 days old (hatching date is taken as day 0). Individuals were identified whether they were ringed the year before or ringed newly; their body mass and tarsus length were measured. Daily ambient temperatures for the period of the breeding season 2010 were obtained from a meteorological station located close to the study site (Zollikofen, Bern, Switzerland).

In 2011, to test for the functional significance of partial incubation during the laying period, we visited the nests daily and numbered each egg according to laying order, and on a daily basis replaced each new egg with a dummy egg. The collected eggs were stored in the lab in a climatic chamber at 10 °C and 60% humidity, each clutch inside a small wooden box containing nest material from the nest of origin. This storage temperature seems to be optimal for maintaining the viability of eggs (Rutkowska and Cichon 2005) because it prevents embryonic development (Lord et al. 2011) and reduces bacterial growth (Fasenko 2007). The chosen humidity mimics the natural humidity in the nest-boxes during the breeding period (McComb and Noble 1981). A total of 31 clutches were randomly assigned to 2 treatments. To simulate partial incubation during the laying period as observed in 2010, eggs of 16 experimental clutches were put into an incubator at 30 °C for 1 h every evening by starting with the first laid egg the first evening and then adding each subsequently laid egg in a clutch to the incubation treatment. Eggs of 15 control clutches were placed in the unheated incubator for 1 h every evening using an identical procedure. We used the same incubator for control and experimental clutches but alternated the sequence of the 2 temperature regimes. Eggs were turned to mimic the natural behavior of females (Derksen 1977; Haftorn 1979; Gee et al. 1995; Deeming 2002). Each egg was turned twice a day (morning and evening) by rotation over its width and length over 360°. Each egg rotation was repeated at least 8 times (blue tits apply around 8 turns per hour, Deeming 2002). At the same time, we followed the nests of origin in the woods to determine the first day of full incubation. The first day of incubation was taken as the day when all eggs in clutch were uncovered and warm to the touch. On the second day of full incubation, eggs were returned from the lab to their nest of origin. Clutch size and laying date did not differ between experimental and control clutches ($\chi^2_{1,29} = 0.07$, $P = 0.79$; $F_{1,29} = 0.83$; $P = 0.37$, respectively).

Hatching date (called day 0) was defined as the day the first nestling of the clutch hatched, and all later measurements of nestlings and parents are in reference to this day. After the first nestling hatched, nests were visited twice a day (morning and evening) to determine hatching spread, number of hatchlings, and nestling body mass at hatching using a portable electronic scale to the nearest 0.01 g. Each nestling was individually marked on the day of hatching by plucking specific combinations of tuft feathers from the head, back, or wings and was thus individually recognizable until old enough to be ringed permanently. On day 9, birds were ringed with an individually numbered aluminum ring (Swiss Ornithological Station, Vogelwarte, Sempach). On days 2 and 14, all nestlings were weighed with a portable electronic scale to the nearest 0.01 g. On day 14, nestling tarsus and wing lengths were measured to the nearest 0.1 mm with a caliper and a ruler, respectively. Adults were caught when nestlings were 12 days old (24 females and 17 males coming from 29 nests). Individuals were identified whether they were ringed the year before or ringed newly, and their body mass, tarsus, and wing lengths were measured. After day 16, nests were checked daily to determine the number of fledged young and fledging date.

Statistical analyses

Statistical analyses were done using R, version 2.14 (R Development Core Team 2010). We used generalized linear model (GLM) with binomial distribution of errors to test whether female body condition influences the probability that partial incubation above physiological zero temperature (25 °C) occurs during laying period. Model included probability that eggs were incubated above 25 °C as binary response variable (partial incubation above 25 °C vs. partial incubation below 25 °C) and residuals from the model of female body condition as explanatory variable. Laying date and clutch

size were included in the model as covariates. Female body condition was included by adding nestling tarsus length as a predictor variable to the analyses of body mass in a GLM with normal error distribution (Freckleton 2002). GLM with binomial distribution of errors was used to test whether daily ambient temperature during laying period influences the probability of partial incubation above 25 °C in a clutch. Partial incubation above or below physiological zero temperature (25 °C) was included as a binary response variable, daily ambient temperature during laying was included as explanatory variable, whereas clutch size was included as a covariate.

Duration of incubation was measured as the number of days between the first day of full incubation and the hatching of the first nestling. Hatching spread was estimated in 2 ways: first, as the number of days (h) between the first- and the last-hatched nestling within a brood; and second, as the differences in body mass between the first- and last-hatched nestling on day 2. Variation of the 3 morphological traits (body mass, tarsus, and wing length) was computed as the coefficient of variation for each brood (CV = standard deviation/mean, Sokal and Rohlf 1995) at the different nestling ages (i.e., days 2 and 14 for body mass and day 14 for tarsus and wing lengths). The CV is statistically more convenient than variance because it is independent of the mean value of the trait (Sokal and Rohlf 1995).

We used GLMs with normal errors to analyze differences among treatments in brood size at day 2, the length of incubation, CV in morphological traits, parent's body condition (body mass corrected by tarsus length), the length of the nestling period, and hatching spread (the difference in body mass between first- and last-hatched nestlings on day 2). GLM with Poisson errors was used to analyze hatching spread (the number of days between the first- and the last-hatched nestling within a brood). Hatching success (the number of hatched eggs over clutch size) and whole-brood fledging success (the number of fledged nestlings over number of hatched nestlings in a brood) were analyzed using GLM with binomial distribution of errors. All initial models included treatment as an explanatory variable.

Nestling morphological traits on day 14 (body mass, tarsus length, and wing length) were analyzed using generalized linear mixed models (GLMM, package nlme, Pinheiro and Bates 2000). In these models, treatment was included as a fixed effect and brood identity as a random effect to correct for genetic and environmental correlations within broods.

In all the analyses, we included laying date as a covariate for seasonal effects (Verhulst and Nilsson 2008). We included clutch size as a covariate in the analyses of the length of incubation, hatching success, hatching spread, brood size on day 2, and parental body condition. We included brood size on day 2 as a covariate in the analyses of posthatching variables.

A stepwise backward selection procedure was used to remove effects with $P > 0.10$ starting with exclusion of interactions. Model selection was done using maximum likelihood estimation, whereas parameter estimates of mixed models with normal errors were obtained via restricted maximum likelihood (Pinheiro and Bates 2000). Fixed effects were tested for significance using 2-tailed type II F or χ^2 tests (Fox 2002). Differences in significant terms were investigated using post hoc t-tests.

RESULTS

Partial incubation during laying

Partial incubation of eggs during the laying period from the first to the last egg was observed in all nests (e.g., Figure 1 representing hourly mean temperature readings from data loggers). After the first egg laid, 10 out of 30 females showed partial incubation early in the evening, an additional 7 females after the second egg, 8 more females after the third egg, and all females thereafter. Temperatures above the physiological zero thresholds (25 °C) increased from around 45 min after the first egg to an average of 560 min toward the end of egg laying (Figure 1).

Female body condition had no influence on the probability of partial incubation above physiological zero temperature (residuals from the model of female body condition: $\chi^2_{1,11} = 0.006$, $P = 0.94$; clutch size: $\chi^2_{1,10} = 0.90$, $P = 0.34$; laying date: $\chi^2_{1,9} = 0.30$, $P = 0.58$). Daily ambient temperature during the laying period did not influence the probability of partial incubation above 25 °C in a clutch (ambient temperature: $\chi^2_{1,28} = 0.24$, $P = 0.63$; clutch size: $\chi^2_{1,27} < 0.001$, $P = 0.98$).

Eggs in the laying order differed in incubation time above 25 °C (i.e., total time during day and night before the start of the full incubation; Figure 2). First-laid eggs in the laying sequence are in total partially incubated for relatively longer times (mean time of incubation for the first-laid egg ± 1 SE: 29.87 h ± 3.39; mean time of incubation for the last-laid egg ± 1 SE: 10.82 h ± 2.03; Figure 2).

Effect of partial incubation on breeding parameters and offspring growth

Simulation of partial incubation of eggs in the lab did not later in situ affect the length of full incubation by females (treatment: $F_{1,27} = 0.04$, $P = 0.83$; clutch size: $F_{1,28} = 0.68$, $P = 0.42$; laying date: $F_{1,29} = 5.74$, $P = 0.02$; mean ± 1 SE for control clutches: 13.67 days ± 0.15; mean ± 1 SE for "partially incubated" clutches: 13.81 days ± 0.14). Hatching success did not differ among treatments (treatment: $\chi^2_{1,29} = 0.008$, $P = 0.93$; clutch size: $\chi^2_{1,28} = 0.37$, $P = 0.54$; laying date: $\chi^2_{1,28} = 0.11$, $P = 0.74$; for control clutches: 82%; for "partially incubated" clutches: 84%) and was also not different from the hatching success of a nonmanipulated neighboring great tit population (hatching success: 84%). Hence, brood size after hatching of all nestlings was similar in the 2 treatments (treatment: $F_{1,27} = 0.07$, $P = 0.79$; clutch size: $F_{1,29} = 7.16$, $P = 0.01$; laying date: $F_{1,28} = 0.64$, $P = 0.43$; mean ± 1 SE for control clutches: 6.40 nestlings ± 0.37; mean ± 1 SE for "partially incubated" clutches: 6.31 nestlings ± 0.36).

Hatching spread, that is, the time in days between the hatching of the first and the last nestling of a brood, was not affected by experimental partial incubation (treatment: $\chi^2_{1,29} = 2.19$, $P = 0.14$; clutch size: $\chi^2_{1,28} = 0.002$, $P = 0.96$; laying date: $\chi^2_{1,27} = 0.30$, $P = 0.58$; median hatching spread in clutches with partial incubation = 1 days; min. = 0; max. = 2; median hatching spread in control clutches = 1 day; min. = 0; max. = 2; mean hatching spread in hours: clutches with partial incubation: 7.69 h ± 1.14; control clutches: 10.04 h ± 1.31) and in fact pointed in the opposite direction than expected, given that on average the summed time of experimental partial incubation was 7.52 h ± 1.50. A similar pattern was observed for hatching spread defined as the difference between body mass of first- and last-hatched nestling on day 2 (treatment: $F_{1,29} = 3.05$, $P = 0.091$; laying date: $F_{1,27} = 0.12$, $P = 0.73$; brood size on day 2: $F_{1,28} = 0.42$, $P = 0.52$; mean differences in body mass ± 1 SE—control clutches: 1.67 g ± 0.12; "partially incubated" clutches: 1.32 g ± 0.15; body mass range—control clutches: (mean) 2.66 g ± 0.08, 0.98–4.44 g; "partially incubated" clutches: (mean) 2.73 g ± 0.07, 1.24–4.40 g). On day 2, the CV in body mass tended to be lower in clutches with partial incubation (Table 1a and Figure 3). However, on day 14, there was no difference in CV of body mass and size between clutches with partial incubation and control clutches (Table 1b–d). Mean body mass and size of nestlings 14 days after hatching did not differ among treatments (Table 2a–c).

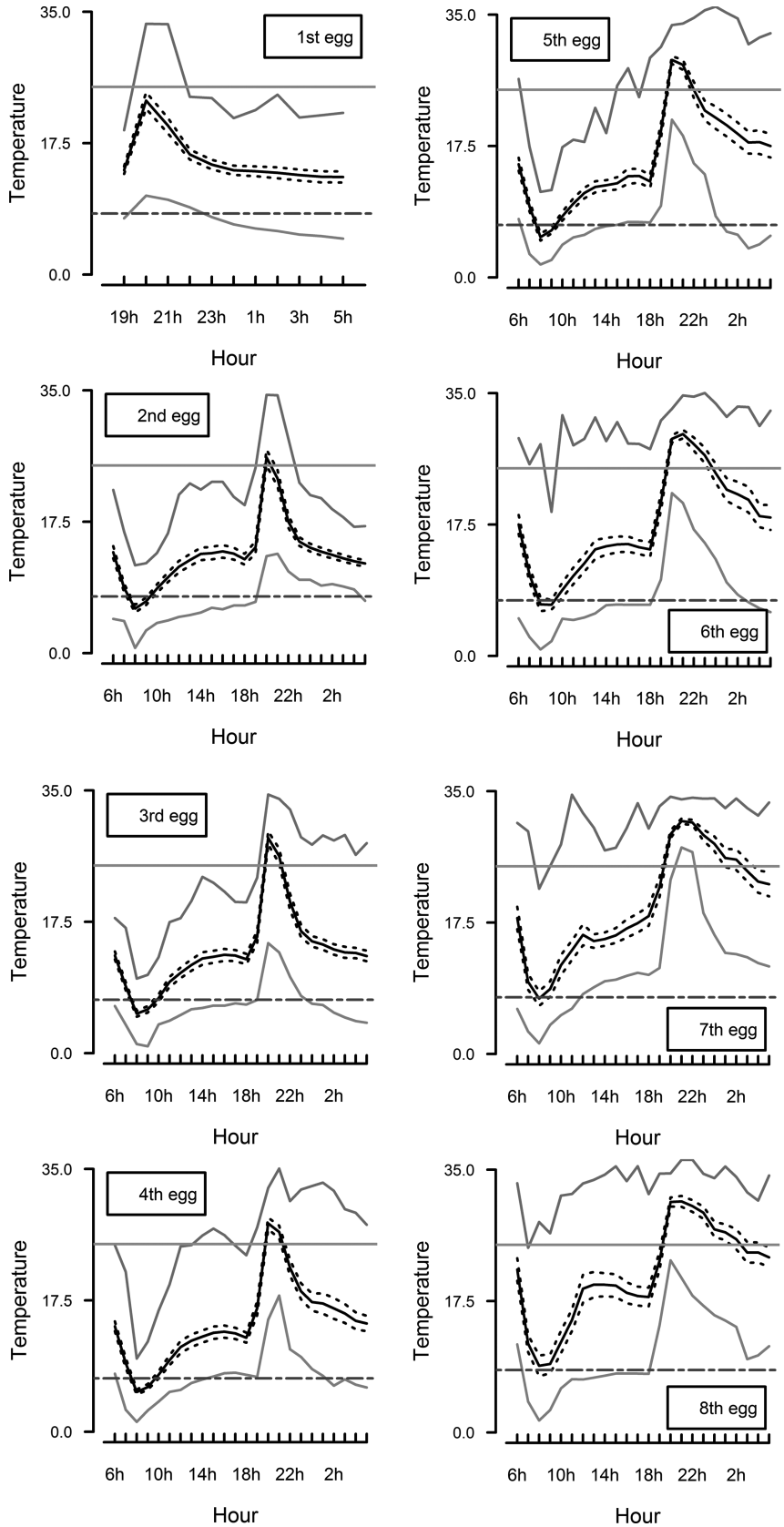

Figure 1
Temperatures recorded during the egg-laying period in great tits for N = 30 nests. Black solid lines indicate the mean temperature by night hour on the days the first and the subsequent eggs were laid and black hatched lines standard errors. The dark gray lines indicate the maximum (upper curve lines) and light gray lines the minimum temperature (bottom curve lines). The gray solid horizontal lines indicate the critical physiological zero temperature (25 °C) for embryo development and gray hatched horizontal lines daily ambient temperature.

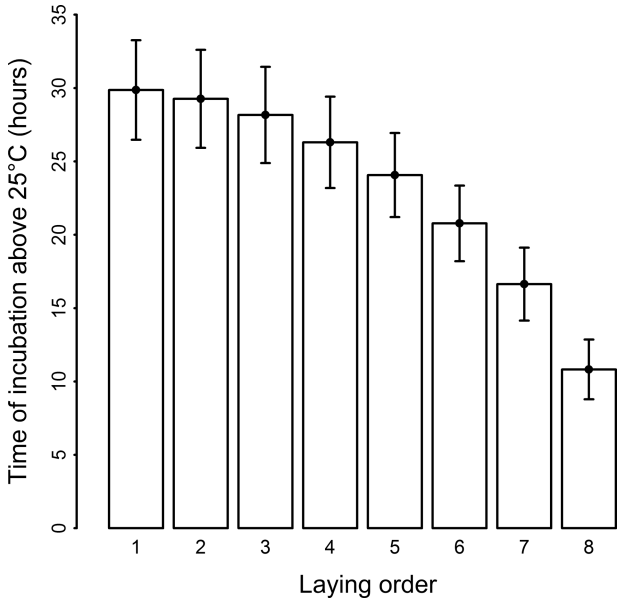

Figure 2
Total incubation time (during day and night) in hours (±SE) of great tit eggs during the laying period and before full incubation started in 2010. Sample size: $N = 30$ nests.

The length of the nestling period did not differ significantly among treatment groups (treatment: $F_{1,25} = 0.12$, $P = 0.73$; brood size: $F_{1,26} = 0.42$, $P = 0.52$; laying date: $F_{1,27} = 1.21$, $P = 0.28$; mean ± 1 SE for control clutches: 19.31 ± 0.30; mean ± 1 SE for "partially incubated" clutches: 19.25 ± 0.27). Whole-brood fledging success, that is, number of nestlings fledged over the number hatched, was also not significantly different between partially incubated and control clutches (treatment: $\chi^2_{1,29} = 1.23$, $P = 0.27$; brood size: $\chi^2_{1,28} = 0.54$, $P = 0.46$; laying date: $\chi^2_{1,27} = 0.01$, $P = 0.92$; whole-brood fledging probability for control clutches = 79%; for "partially incubated" clutches = 96%).

Table 1
GLM testing the effects of partial incubation on within-brood variation in nestling great tits mass and size

	Estimates ± 1 SE	F	df	P
(a) CV in body mass on day 2				
Intercept	0.24 ± 0.02	11.92	1,26	<0.001
Treatment[a]	**−0.06 ± 0.03**	**4.22**	**1,26**	**0.050**
Brood size on day 2	−0.01 ± 0.01	1.98	1,25	0.17
Laying date	−0.002 ± 0.004	0.13	1,24	0.72
(b) CV in body mass on day 14				
Intercept	0.06 ± 0.008	7.70	1,19	<0.001
Treatment[a]	−0.01 ± 0.01	0.83	1,19	0.37
Brood size on day 2	0.002 ± 0.004	0.26	1,18	0.61
Laying date	0.0004 ± 0.002	0.05	1,17	0.82
(c) CV in tarsus length on day 14				
Intercept	0.009 ± 0.01	0.73	1,19	0.48
Treatment[a]	−0.0003 ± 0.006	0.002	1,17	0.96
Brood size on day 2	0.002 ± 0.002	0.91	1,19	0.35
Laying date	0.0004 ± 0.0009	0.23	1,18	0.64
(d) CV in wing length on day 14				
Intercept	0.18 ± 0.06	2.82	1,18	0.011
Treatment[a]	−0.02 ± 0.009	3.38	1,18	0.082
Brood size on day 2	−0.0003 ± 0.003	0.009	1,17	0.92
Laying date	−0.002 ± 0.001	3.50	1,18	0.077

Significant terms in the model are in bold.
[a]Relative to the clutches with partial incubation.

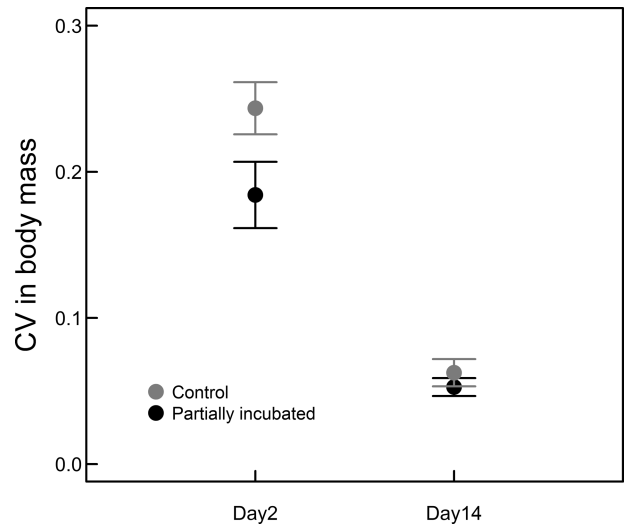

Figure 3
CV of body mass (±SE) of nestling great tits in control (gray dots) and partially incubated clutches (black dot) on days 2 and 14 after hatching. Sample size: $N = 30$ nests.

Table 2
GLMM testing the effects of partial incubation on body mass, tarsus, and wing length of 14-day-old nestling great tits

	Estimates ± 1 SE	F	df	P
(a) Body mass				
Intercept	18.15 ± 1.11	16.36	1,144	<0.001
Treatment[a]	0.81 ± 0.47	3.01	1,26	0.094
Brood size on day 2	**−0.51 ± 0.17**	**9.33**	**1,26**	**0.005**
Laying date	0.02 ± 0.07	0.11	1,25	0.74
(b) Tarsus length				
Intercept	16.76 ± 1.72	9.73	1,144	<0.001
Treatment[a]	0.38 ± 0.25	2.40	1,26	0.13
Brood size on day 2	−0.08 ± 0.08	1.09	1,25	0.31
Laying date	0.06 ± 0.04	2.70	1,25	0.11
(c) Wing length				
Intercept	44.31 ± 1.15	38.63	1,144	<0.001
Treatment[a]	2.06 ± 1.54	1.78	1,27	0.19
Brood size on day 2	−0.20 ± 0.54	0.14	1,25	0.71
Laying date	0.12 ± 0.23	0.29	1,26	0.59

Significant terms in the model are in bold.
[a]Relative to the clutches with partial incubation.

Effect of partial incubation on parents

Female body condition did not differ among treatments (treatment: $F_{1,19} = 0.03$, $P = 0.85$; tarsus length: $F_{1,21} = 7.34$, $P = 0.01$; clutch size: $F_{1,20} = 0.73$, $P = 0.40$; laying date: $F_{1,21} = 6.26$, $P = 0.02$). Same pattern was observed for male body condition (treatment: $F_{1,13} = 2.64$, $P = 0.13$; tarsus length: $F_{1,14} = 5.33$, $P = 0.037$; clutch size: $F_{1,12} = 0.91$, $P = 0.36$; laying date: $F_{1,11} = 0.03$, $P = 0.87$).

DISCUSSION

The main aim of this study was 1) to investigate the occurrence and extent of partial incubation and 2) to evaluate experimentally its potential functions. In a first step, we measured the clutch temperature over the whole laying period using data loggers, and in a second step, we simulated these observed patterns in the lab. Overall, we found that partial incubation occurred in all nests and that egg temperature was raised above physiological zero temperature where embryo

development occurs (25 °C). We showed that experimental simulation of partial incubation did not affect any measured breeding parameters or offspring growth and parental body condition. Partial incubation, despite temperatures above physiological zero, did not increase hatching asynchrony or size asymmetries among offspring within the brood, which are among the few functions most often discussed.

Given that partial incubation is common among many bird species, its extent and causes are not well understood and little is known about the temperatures attained (Wang and Beissinger 2011). Our data on egg temperature during the laying period clearly show that partial incubation raises egg temperature for considerable time periods above the critical temperature for embryo growth. This result corroborates the findings of 2 previous studies on the same species (Haftorn 1981; Lord et al. 2011), but contradicts another one (Pendlebury and Bryant 2005) that showed that female great tits used the nest cup to roost at night but did not raise egg temperature to the physiological zero for embryo development. Studies on other species have also shown mixed results (reviewed in Wang and Beissinger 2011). This, on the one hand, may be due to the methods used for data collection (Wang and Beissinger 2011), but on the other hand, it may genuinely reflect genetic, physiological, and environmental factors involved in the emergence of partial incubation (Wiebe et al. 1998; Hanssen et al. 2002; Wang and Beissinger 2011). Therefore, more studies with accurate methodology seem still needed to assess patterns of partial incubation and their intraspecific variation.

Previous studies in other species found the probability that females start to incubate their eggs before clutch completion increased with increasing temperature in nest-boxes (Veiga and Vinuela 1993; Ardia et al. 2006) and that the quality of females may affect incubation investment (Ardia and Clotfelter 2007). Our results, however, show no influence of daily ambient temperatures on the probability of partial incubation above 25 °C. Moreover, the probability for partial incubation above 25 °C of first laid eggs was independent of female quality in our study. Although it seems that our results contradict previous findings, it has to be mentioned that ambient temperatures may differ from those inside the nest-boxes (McComb and Noble 1981) and thus may lead to different results. Our result regarding the effect of female quality may be taken with caution because it is based on 13 females only.

It has been suggested that partial incubation with heat transfer above the physiological zero temperature is induced to reduce the risk of hatching failure (Arnold et al. 1987), to increase hatching asynchrony (Kennamer et al. 1990; Lord et al. 2011), and/or to reduce the length of full incubation or nestling period (Fasenko 2007). However, an experimental simulation of partial incubation in our study does not support the idea of a specific role of partial incubation in great tits. First, we did not find any differences in hatching success of eggs among experimental treatments. Second, against the predictions, we also did not find the differences in hatching spread neither represented by a difference in days between first- and last-hatched nestling within a brood nor as a difference in body mass between first- and last-hatched nestlings on day 2. Moreover, within-brood variation in body mass on day 2 tended to be lower in clutches with partial incubation, which is even opposite to the predicted effect (Laaksonen 2004). No difference in body mass and size of 14-day-old nestlings was found among treatments. Lastly, there was no difference in length of full incubation and length of the nestling period between clutches with partial incubation and control ones.

Wilson (1991) suggested that tissues of developing embryo may have different threshold temperatures for development resulting in uneven or unsynchronized growth if the embryo is kept for a certain period between 27 and 35 °C. Decuypere and Michels (1992) suggested, however, that the threshold temperatures for development might be different according to the part of the incubation period studied. It is therefore possible that the temperature of 30 °C used in our study could be insufficient to induce embryo development at the early period of egg laying.

Our experimental study, therefore, does not lend support to the hypothesis of alterations of hatching patterns, which is one of its most often discussed functions (Grenier and Beissinger 1999). It suggests that the onset of full incubation while eggs are still being laid might be a more potent mechanism for inducing hatching asynchrony than partial incubation, as also suggested by other studies (Stoleson and Beissinger 1997). In addition, hatching patterns may be more strongly influenced by other factors such as egg content, clutch size, female quality, and incubation behavior (Veiga and Boto 2000; Eising et al. 2001).

Our study calls for alternative explanations regarding the function of partial incubation. A raise of egg temperatures can activate lysozymes, an enzyme abundant in albumen with antimicrobial properties against Gram-positive bacteria (Wellman-Labadie et al. 2008). Microbial growth can occur both on and in the egg (Cook et al. 2003). On the other hand, it has been suggested that the viability of unincubated eggs declines over time and partial incubation may reduce the risk of hatching failure because embryos that have passed the early developmental stages can be more resistant to environmental fluctuations ("the egg viability hypothesis," Arnold et al. 1987; Veiga 1992; Stoleson and Beissinger 1999; Ardia et al. 2006). Furthermore, partial incubation of eggs may serve to protect the eggs against predators (Wiebe and Martin 1998), brood parasites (Clotfelter and Yasukawa 1999), or a takeover by other breeders (Stoleson and Beissinger 1995; Beissinger et al. 1998). However, it would not explain why temperatures are then raised above physiological zero. Future research should be designed to address these alternatives.

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